

## X-RAY IMAGING MICROSCOPE FOR CANCER RESEARCH

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### ABSTRACT

The NASA technology employed during the Stanford/MSFC/LLNL Rocket X-Ray Spectroheliograph flight established that doubly reflecting, normal incidence multilayer optics can be designed, fabricated and used for high resolution x-ray imaging of the Sun. Technology developed as part of the MSFC X-Ray Microscope program, demonstrated that high quality, high resolution multilayer x-ray imaging microscopes are feasible. Using technology developed at Stanford University and at the DOE/Lawrence Livermore National Laboratory (LLNL), Troy W. Barbee, Jr. has fabricated multilayer coatings with near theoretical reflectivities and perfect bandpass matching for a new rocket-borne solar observatory, the Multi-Spectral Solar Telescope Array (MSSTA). Advanced Flow Polishing has provided multilayer mirror substrates with sub-angstrom (rms) smoothness for our astronomical x-ray telescopes and x-ray microscopes. The combination of these important technological advancements has paved the way for the development of a *Water Window Imaging X-Ray Microscope* for cancer research.

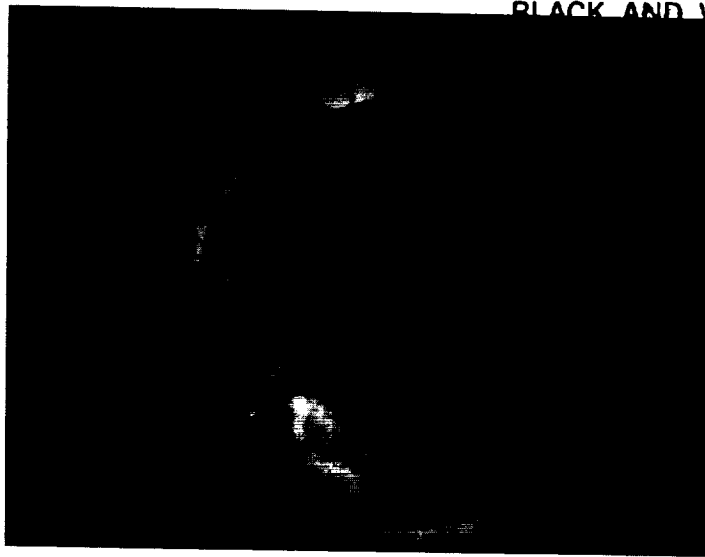
This instrument is a doubly reflecting multilayer coated x-ray microscope configured to operate within the "water window," a narrow regime of the x-ray spectrum between the K absorption edges of oxygen (23.3 Å) and of carbon (43.62 Å). In this wavelength regime, water is relatively highly transmissive and carbon is highly absorptive, permitting the microscope to delineate carbon based structures within living cells. The development of this high spatial resolution and high contrast capacity to image living cells, in aqueous physiological environments, will afford advantages not available in any conventional microscopes. The Water Window Imaging X-Ray Microscope offers non-invasive strategies for examining living tumor cells without the need of dyes, stains or exogeneous chemicals which produce limiting artifacts. Our theoretical analysis has shown that multilayer x-ray microscopes of the Schwarzschild configuration should achieve spatial resolution in the 100 Å range or better. Such performance could permit direct imagery of cytoskeletal components, membranes, secretory vesicles, endoplasmic reticulum, chromatin, nucleoli and nucleosomes. It should improve diagnosis and greatly benefit experimental studies of tumor cell biology. Advanced versions using aspheric optics and multiple elements may achieve spatial resolution sufficient to resolve DNA and RNA molecules within living tumor cells (the double helix of the DNA molecule is typically only 20 Å wide, but very long). In this paper, we will describe the design of the Water Window Imaging X-Ray Microscope and discuss technological aspects of mirror fabrication, optical assembly, alignment and testing of the instrument. We predict the optical performance and consider applicability of this microscope to studies of cell biology in general and tumors in particular.

## INTRODUCTION

On Oct. 23, 1987 the Stanford/MSFC/LLNL Rocket X-Ray Spectroheliograph<sup>1</sup> was successfully launched on a Nike-boosted Black Brant sounding rocket from the White Sands Missile Range, New Mexico. This flight produced the first high resolution, narrow wavelength band x-ray/EUV images of the Sun (Fig. 1) obtained with normal incidence multilayer x-ray optics. We had previously produced high resolution x-ray images at 44 Å with doubly reflecting multilayer telescopes at the MSFC X-Ray Calibration Facility.<sup>2</sup> These laboratory and solar images constitute dramatic proof that normal incidence multilayer coatings on curved surfaces can produce superb x-ray/EUV images. Photons of wavelengths of 1-100 angstroms (Å) are considered to be x-rays, and 100 - 1000 Å comprise the Extreme Ultraviolet (EUV) regime. The high density solar images we recorded on high resolution, but relatively insensitive, photographic emulsions demonstrated that multilayer coatings can yield excellent x-ray/EUV reflectivities and that the bandpasses of peak reflectivity of the two mirrors of a doubly reflecting multilayer imaging system can be precisely matched.

ORIGINAL PAGE

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**Fig. 1.** High resolution 173 Å image of the Sun produced with a doubly reflecting Cassegrain multilayer x-ray telescope operating at normal incidence.

Normal incidence multilayer x-ray optics are the result of the pioneering work of one of the authors, Troy W. Barbee, Jr. at Stanford University<sup>3</sup> and Eberhard Spiller<sup>4</sup> of the IBM Watson Research Center. Their revolutionary breakthroughs in materials science technology, which permitted the fabrication of stable multilayer x-ray optical coatings, were independently achieved only a little over a decade ago. More recently, while at the Lawrence Livermore National Laboratory (LLNL), Barbee has fabricated multilayer coatings, of unprecedented quality, with near theoretical reflectivities at x-ray/EUV wavelengths. Precise bandpass matching was achieved for the primary and secondary optical elements of telescopes which will be flown by NASA. This payload, known as the Multi-Spectral Solar Telescope Array (MSSTA),<sup>5</sup> is larger than its predecessor and will be launched on a Terrier-boosted Black Brant sounding rocket early in 1991. The 127 mm aperture MSSTA telescopes, each with four times the collecting area of the Cassegrain telescope previously flown, employ Ritchey-Chrétien optical systems (Fig. 2). In these telescopes, the primary and secondary mirrors are hyperboloidal. These are "aplanatic" telescopes, which means that the optical aberration known as coma is zero (to third order at least) and spherical aberration is absent. This design permits the telescopes to produce high spatial resolution images over a wide field of view. Theoretical calculations and laboratory studies of these instruments indicate that these telescopes should produce full-disk solar x-ray/EUV images with spatial resolution as high as 0.1 arc second.<sup>6</sup>

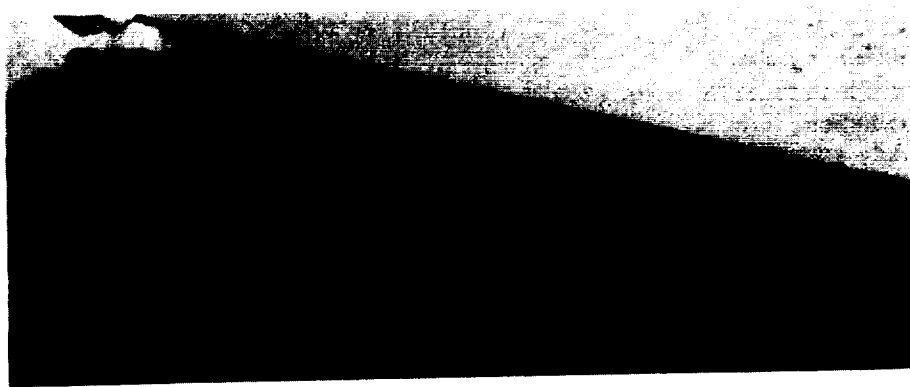


Fig. 2. Completed MSSTA Ritchey-Chrétien multilayer telescopes, using fabrication technology similar to that planned for the Water Window Imaging X-Ray Microscope.

During the development of the MSSTA telescopes, it was also established that the application of the Advanced Flow Polishing technology (pioneered by one of the authors, Phillip C. Baker of Baker Consulting) can yield mirror substrates with sub-angstrom level rms surface smoothness.<sup>7</sup> Based upon these results, a program was initiated at MSFC under the auspices of the Center Director's Discretionary Fund (CDDF) to develop normal incidence magnifying Schwarzschild x-ray microscope optics for coupling X-Ray telescopes to detectors for resolution enhancement. The microscope optics were produced on Zerodur blanks to smoothness of 2-3 Å rms. However, during this program, it was also shown that contoured surfaces with unprecedented rms smoothness in the range of 0.5-0.6 Å could be produced on Hemlite grade Sapphire blanks by Advanced Flow Polishing.

These profoundly important technological advancements in the ability to fabricate high reflectivity, matched bandpass, normal incidence optics operating at 44 Å and the ability to produce the ultra-smooth substrates required by these coatings led us to conclude that a Water Window Imaging X-Ray Microscope is feasible. This instrument affords great promise as a fundamental tool for basic cell biology and cancer research.

### MULTILAYER X-RAY OPTICS

Multilayer x-ray mirrors are essentially synthetic Bragg crystals that can be contoured to a figured surface. (For a detailed description of Multilayer X-Ray Mirrors see Barbee<sup>8</sup>). They are fabricated by the accurate deposition on an ultra-smooth substrate of a coating consisting of a stack of many alternating layers of high atomic number (high-Z) diffractor material separated by layers of a low-Z spacer material. The layers must be very uniform and of precisely repeatable thicknesses  $d_1$  and  $d_2$ , respectively. Since this multilayer coating constitutes a synthetic Bragg crystal, x-ray reflection occurs by the process of Bragg diffraction. When slight refraction effects are ignored, the wavelength at which the peak of the reflectivity occurs is given by the Bragg relation:  $n(\lambda) = 2D \sin(\theta)$ , where  $D = d_1 + d_2$  and  $\theta$  is the angle at which the radiation strikes the mirror, as measured from the mirror surface. For mirrors operating at normal incidence ( $\theta = 90^\circ$ ), the equation becomes:  $\lambda = 2D$ , where  $\lambda$  is the wavelength of peak reflectivity of the first order Bragg diffracted light. Since  $D$  is the sum of the two layer thicknesses, a multilayer coating, designed to reflect 44 Å x-rays, could be produced as a stack of alternating 11 Å thick layers of a high-Z material (such as tungsten carbide) separated by 11 Å thick layers of a low-Z spacer material (such as carbon). Considering the dimensions involved, it is clear that these layers are only a few atoms thick. Furthermore, the diffracting layers must be coplanar and uniform. Since the layers follow the contour of the substrate upon which they are deposited, there are very stringent requirements on the smoothness and uniformity of multilayer optics substrates. This is especially important for layers with thicknesses in the 6 - 11 Å regime, which are necessary for systems designed to operate at normal incidence in the water window. For the multilayer coating to be an effective reflector, there must be many layers in the stack and all must be of the same thickness to a very high degree.

of accuracy. Indeed, by choice <sup>9</sup> of the D spacing and the materials comprising the multilayer coating, it is possible to tailor the coating to reflect very narrow bandpasses at selected wavelengths of x-ray/EUV radiation.

Although only a small fraction of the incident radiation is reflected at each low-Z/high-Z interface, by use of a stack of tens to hundreds of alternating layers in the coating, high reflectivities at normal incidence can be achieved by constructive interference if the layer pairs are deposited with sufficient uniformity. Recently, tests were performed at the Stanford Synchrotron Radiation Laboratory (SSRL) and the National Institute of Standards and Technology (NIST) SURF II Synchrotron on the MSSTA telescopes. These studies revealed that reflectivities approaching 45% at normal incidence were produced by the mirrors operating at 173 Å and 193 Å. For our solar rocket program, optics were fabricated with coatings on both convex and concave superpolished substrates whose reflectivities were peaked for x-ray and EUV radiation in the range  $44 \text{ Å} < \lambda < 335 \text{ Å}$ .

Since multilayer x-ray mirrors reflect x-rays by the phenomenon of Bragg diffraction, only a very narrow bandpass is efficiently reflected wherein the Bragg condition is satisfied. In the water window, multilayer mirrors can achieve spectral resolution ( $\lambda/\Delta\lambda$ ) exceeding 50. This characteristic is of the utmost importance for the development of a *Water Window Imaging X-Ray Microscope*. X-rays of longer or shorter wavelength would seriously degrade the contrast of carbon structures within the cell, which is a major reason why grazing incidence x-ray optics are not well suited as optics for the fabrication of a Water Window X-Ray Microscope. Grazing incidence optics reflect x-rays over a much broader bandpass than the water window. They are also far more sensitive to contaminants and x-ray scattering and suffer more severely from optical aberrations than normal incidence multilayer optics. Because multilayer optics reflect in only a very narrow bandpass of the incident radiation, precise matching of the wavelength at which peak reflectivity occurs from the primary and secondary mirrors is required. If these bandpasses are not accurately matched the net throughput of the instrument will be drastically reduced.

## X-RAY IMAGING IN THE WATER WINDOW

The biological significance of the x-ray water window is due to the fact that water is prevalent in all living cells. The oxygen of the water plays the dominant role for the absorption of soft x-rays. However, the structures that are of the greatest scientific interest (such as organelles, cytoskeletal components, membranes, secretory vesicles, endoplasmic reticulum, chromatin, nucleoli and nucleosomes) are in general comprised of complex molecules (DNA, RNA, proteins, etc.) incorporating large amounts of carbon. The nature of the interaction of x-rays with matter makes it possible to observe these carbon structures with minimal interference from the surrounding water. A sharp discontinuity or "edge" in the absorption spectrum of a material occurs when the energy of the photon is sufficient to ionize electrons from one of the shells or sub-shells of the atoms comprising the material. These edges are designated by the shell or subshell from which the electrons are ejected, i.e. K for the innermost shell,  $L_I$ ,  $L_{II}$ , and  $L_{III}$  for the sublevels of the next shell, etc. The strongest *absorption edges* are the K edges. For wavelengths immediately below the K edge, the absorptivity increases dramatically. Since the x-rays whose wavelength are longward of the K absorption edge do not have sufficient energy to eject a K-shell electron, they are not strongly absorbed by the material, which appears relatively transparent at these wavelengths.

For the purposes of x-ray microscopy, it is significant that the K absorption edge for the element oxygen lies at 23.3 Å and for carbon the K edge is at 43.62 Å. This results in a narrow bandpass in the soft x-ray spectrum between 23.3 Å (oxygen K absorption edge) and 43.62 Å (carbon K absorption edge) called the "water window." In this wavelength regime, water is relatively transparent and carbon is highly absorptive. The opacity of protein and water at these wavelengths has been calculated by London et al.<sup>10</sup> and are shown in Fig. 3. These results show the dramatic difference in the absorptivity of protein and water within the water window. The Water Window Imaging X-Ray Microscope will make it possible to investigate carbon structures (and possibly even the motions of those structures) within the aqueous environment of living cells.

We have, therefore, initiated a program to fabricate an imaging x-ray microscope, utilizing ultra-smooth, Advanced Flow Polished and figured sapphire mirror substrates coated with multilayers,

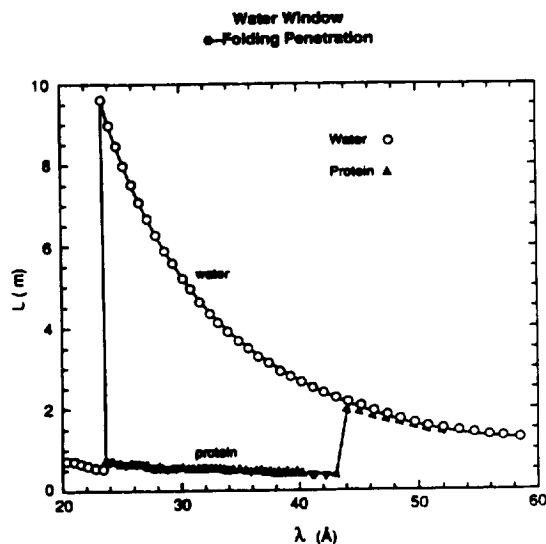


TABLE I. Schwarzschild mirror parameters.

M(x)	R <sub>1</sub> (cm)	R <sub>2</sub> (cm)	s (cm)	d (cm)	Z (cm)	f (cm)
2	105.00	10.00	18.25	98.00	-91.42	5.51
3	58.27	10.00	18.05	48.27	-34.12	6.04
4	45.58	10.00	18.01	35.58	-13.56	6.41
5	40.00	10.00	18.00	30.00	0.0	6.67
10	31.79	10.00	18.02	21.79	48.36	7.29
15	29.69	10.00	18.04	19.69	91.04	7.54
20	28.75	10.00	18.05	18.75	131.49	7.67
30	27.84	10.00	18.06	17.84	213.27	7.80
40	27.40	10.00	18.07	17.40	296.07	7.87
50	27.15	10.00	18.07	17.15	376.42	7.92

Fig. 3. E-Folding penetration of x-rays in the water window. Re-drawn from London *et al.*<sup>10</sup>

with 2D spacing such that  $23.3\text{Å} < 2D < 43.62\text{Å}$ , as is appropriate to reflect x-rays of a narrow bandpass within the water window. At these wavelengths, ultra-high resolution photographic films (i.e. XUV 100 and XUV 649) and photo-resists can be used as the x-ray detector. Since the 649 emulsion affords spatial resolution of 2000 lines/mm when processed for optimum resolution, and a very great dynamic range, it will serve as a primary detector for the instrument. This microscope should be capable of producing high resolution, high contrast images of chromosomes, proteins and other carbon structures within living or freshly killed cells. The *Water Window Imaging X-Ray Microscope* should permit smaller structures to be resolved than is currently possible with visible light or fluorescence microscopy. By obtaining sequential images using high repetition rate laser plasma x-ray sources, it may be possible to investigate motions of genetic material, proteins and other structures within living cells.

### OPTICAL CONFIGURATION OF THE MULTILAYER X-RAY MICROSCOPE

The exciting results which we obtained with normal incidence multilayer x-ray telescopes encouraged us to continue our efforts to develop an aplanatic imaging x-ray microscope utilizing multilayer optics in the Schwarzschild configuration. Normal incidence multilayer x-ray optics for use in scanning x-ray microscopes were studied by Spiller<sup>11</sup> using elliptical mirrors. Trail and Byer<sup>12</sup> fabricated a scanning microscope and Lovas *et al.*<sup>13</sup> employed a Schwarzschild system for laser fusion research. Our prior studies of Schwarzschild multilayer microscopes were constrained to the development of systems for which high smoothness spherical laser mirrors were available as "off-the-shelf" components. Suitable spherical substrates had been purchased from General Optics of Moorepark, CA, and were used for the telescopes flown on Oct. 23, 1987. However, during the development of the MSSTA, Baker Consulting produced hyperboloidal optical substrates of ultra-high smoothness (1-3 Å rms). His fabrication methods can also yield spherical or aspheric substrates which are ideal for x-ray microscopes. High resolution aplanatic imaging x-ray microscopes configured from low x-ray scatter normal incidence multilayer optics should find important applications in many areas, including laser fusion research, x-ray lithography, materials science, astronomy, genetic engineering, virology and bacteriology, as well as fundamental cell biology and cancer research.

We have designed and analyzed several Schwarzschild x-ray microscope configurations. Diffraction analysis indicates better than 200 Å spatial resolution in the object plane for up to a 1 mm field of view can be achieved with 125 Å radiation. Since the diffraction limit scales with the wavelength, when the microscope is used with 37 Å radiation (within the water window) spatial resolution well below 100 Å may be realized. We are currently fabricating 20X and 30X normal incidence multilayer x-ray microscopes of 1.35 meter overall length. An aplanatic x-ray microscope using two spherical mirrors can be constructed by imposing the Schwarzschild condition on the selection of the mirror radii. The Schwarzschild condition can best be understood by referring to Fig. 4.

The mirror surfaces  $S_1$  and  $S_2$  are concentric spherical surfaces of radii  $R_1$  and  $R_2$ , respectively. A complete discussion of the ray trace analysis of a Schwarzschild microscope configured for normal incidence multilayer applications was presented by Hoover et al.<sup>14</sup> and Shealy et al.<sup>15</sup>. The Schwarzschild condition for an aplanatic, two mirror imaging system can be expressed:

$$\frac{R_2}{R_1} = 1.5 - \frac{R_2}{Z_o} \pm \left[ 1.25 - \frac{R_2}{Z_o} \right]^{1/2}, \quad (1)$$

where the "+" sign is used in Eq. 1 for magnifications greater than 5, and the "-" sign is used for magnifications less than 5. Hoover et al.<sup>14</sup> have summarized the Schwarzschild design equations and presented the dependence of the rms blur circle radius as a function of the object height, image plane location, mirror tilts, and decentration for a 10x microscope with a total length of 1.41 m. As the magnification increases so does the overall length of the microscope. The parameters for systems varying in magnifications from 2X to 50X have been computed and are given in Table 1. The mirror substrates which we have fabricated were selected for 20X and 30X Schwarzschild systems.

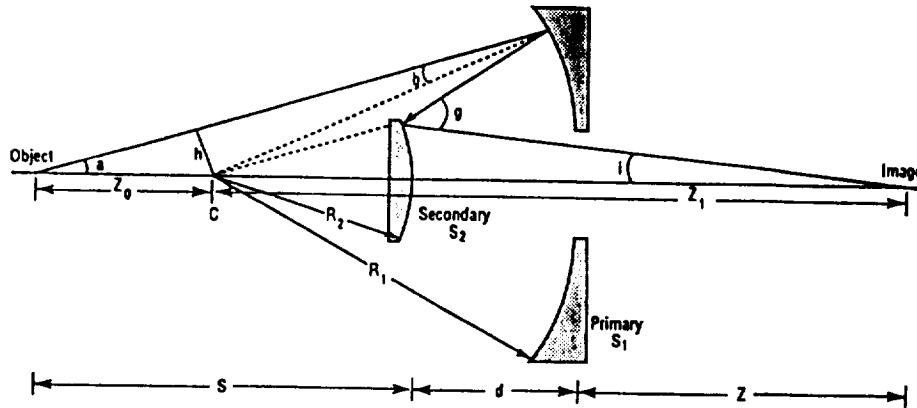


Fig. 4. Schwarzschild configuration for an aplanatic normal incidence x-ray microscope.

We have calculated the spatial resolution, transmission losses due to vignetting, and off-axis performance for these microscopes. These calculations imply that in order to take advantage of this high spatial resolution, photographic emulsions capable of achieving 0.78 micron spatial resolution will be required. This implies the need for films capable of resolving better than 1300 lines per mm over a 20 mm diameter regime. We have established that the Kodak 649 emulsion has the required spatial resolution and is sensitive over the soft x-ray/EUV portions of the spectrum.<sup>16</sup>

Even higher resolution may be achieved by the use of aspheric optics. We have carried out a theoretical design and analysis of aspheric x-ray microscope configurations, which yield far better resolution over a wider field of view than is possible with the Schwarzschild configuration<sup>17</sup>. Results of the analysis of the the aspherical 20X microscope also reveal that superior off-axis performance due to reductions in coma can be achieved.

## FABRICATION OF THE X-RAY MICROSCOPE

The 20X and 30X Schwarzschild x-ray microscopes are now being fabricated utilizing much of the technology implemented in the MSSTA program. Baker Consulting has fabricated mirror substrates from both Zerodur and Hemlite grade Sapphire. It is very important that the mirror substrate material have the ability to be polished to an ultra-smooth finish, but also have low thermal expansion coefficient. Although sapphire has somewhat higher thermal expansion properties than Zerodur, it can be polished to phenomenal smoothness. In addition the sub-surface condition of the mirror substrate must be considered as a possible factor in the performance due to possible stress relaxation during coating or from externally applied force either thermal or mechanical. It has

been demonstrated, during the fabrication of sapphire surfaces, that the use of the Advanced Flow Polishing technique has produced a zero sub-surface damage condition as measured with Rutherford backscatter techniques by General Ionex Corp.

The primary mirrors were fabricated as concave spheres of 8 cm outside diameter. They have a radius of curvature of 23 cm and a central hole diameter of 2.2 cm. The convex spherical secondaries are of 2 cm diameter with an 8 cm radius of curvature. The Sapphire optical surfaces were polished to 0.5-0.6 Å rms surface smoothness and surface figure accuracy better than  $\lambda/10$  when tested with visible light. The mirror substrate smoothness was measured using a Zygo profilometer and the completed optical systems were tested by interferometric techniques at visible wavelengths to insure that the precise optical figure of the elements was obtained. Final performance testing of the completed x-ray microscope assembly will be carried out by producing images of microscopic structures with the instrument at x-ray wavelengths. These tests require the use of ultra-high resolution photographic emulsions and high intensity x-ray sources. Initial studies will be carried out using the laser fusion plasma produced by the OMEGA Facility of the University of Rochester, and with x-ray/EUV emission generated by the NIST SURF II or the Stanford SSRL synchrotron facilities. All microscope structures have been designed and are currently being fabricated. The mount structures are of stainless steel, with a conical configuration so as to be compatible with the beam constraints of the OMEGA Facility. The x-ray microscope currently being fabricated is shown in Fig. 5.

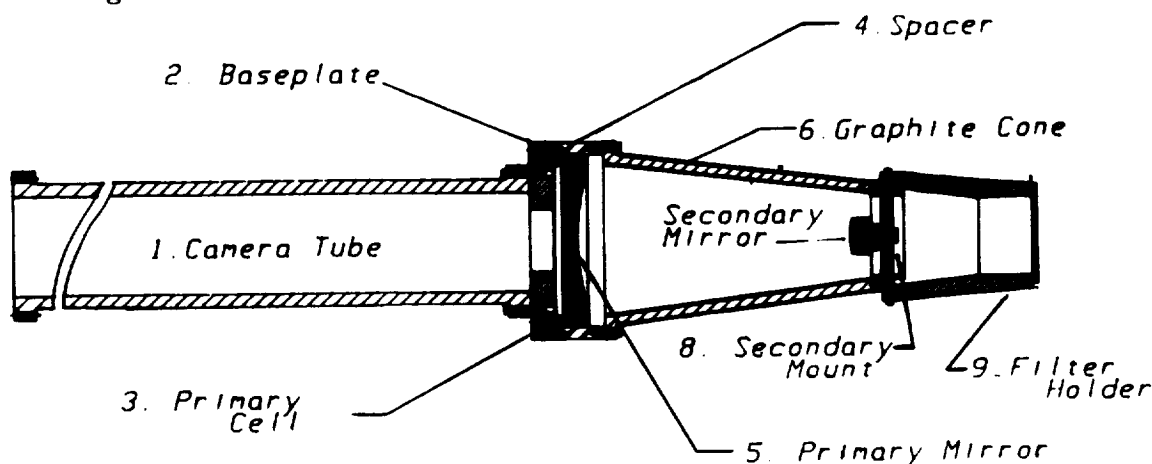


Fig. 5. Structural configuration of the Water Window Imaging X-Ray Microscope.

The microscope tube structures are being fabricated by filament winding methods using AS4-12K graphite fiber with an HBRF55A epoxy resin matrix. Longitudinal fibers are applied to increase stiffness and to produce microscope tube structures with near zero coefficient of thermal expansion. This is the same technique used to fabricate the tube structures for the 127 mm diameter telescopes for MSSTA. Thin film filters of 1500 Å aluminum on a nickel support mesh have been fabricated by the Luxel Corp. of Friday Harbor, Washington. These will be used as both specimen supports and visible light rejection filters. The microscope camera adapters accommodate either the 35 mm Canon T-70's, which were flown on the Rocket X-Ray Spectroheliograph, or the 70 mm Pentax 645 MSSTA cameras. Primary data recording will be on photographic film, utilizing experimental XUV-100, 101-07, and XUV 649 emulsions.

### MEASURED PERFORMANCE OF X-RAY MICROSCOPE OPTICS

We have fabricated the optical components for 20X and 30X Schwarzschild x-ray microscopes using Zerodur and sapphire. The mirrors were flow polished, as noted above, and a surface smoothness of 1 Å rms was achieved on Zerodur and 0.5-0.6 Å on sapphire. The surface figure achieved on the mirrors was far better than the  $\lambda/10$  specification. In fact, the completed optical systems were tested interferometrically and found to have superb performance characteristics, with rms wavefront

errors less than  $\lambda/100$ . Figure 6a. shows the measured geometric radial energy distribution of the a 20X Zerodur Schwarzschild mirror system. It can be seen that 90% of the energy is contained within 0.12 Airy disk radii. The superb point spread function of this system, as determined by interferometric analysis of the optics, is shown in Figure 6b.

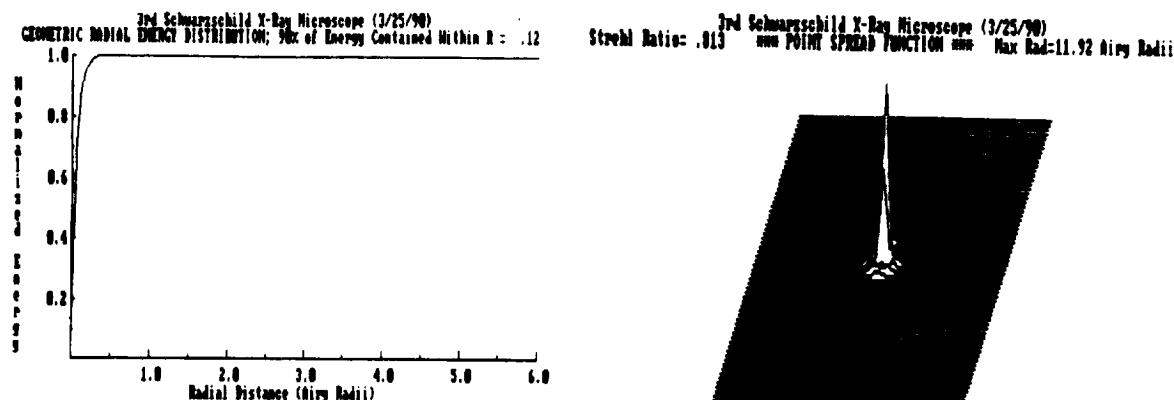


Fig. 6. Multi-pass interferometer measurements of (a.) the geometric radial energy distribution and (b.) the Point Spread Function for the 20X Schwarzschild microscope.

The first Schwarzschild microscope prototype x-ray optics are being coated with multilayers for operation at 130-135 Å. Normal incidence reflectivities better than 55% have been experimentally achieved from multilayers on concave surfaces in this wavelength range. Based upon the theoretical analysis and measurements of the optical characteristics of the completed mirrors which we have performed, we anticipate a spatial resolution of 300-400 Å in the object plane should be obtainable with a Schwarzschild microscope operating at an initial test wavelength of 130-135 Å. In the water window with mirrors of the same design coated to reflect radiation of 36-40 Å wavelength, significantly better spatial resolution should be realized. After final assembly and optical alignment of the multilayer microscope at MSFC, x-ray tests and utilization of the instrument for imaging applications will begin at SSRL, SURF II and the OMEGA Facility. The imaging and assessment phase at synchrotrons and the laser fusion facility could be accomplished within a year.

The requirements for the performance of the optical systems have become more exacting, driven by the high quality of the multilayer coatings and the demands of the imaging requirements at the shorter wavelengths. This has led to our use of special optical testing techniques for the measurement of the surfaces and the contours of the microscope and telescope optics. The standard interferometric analysis at the longer wavelengths (i.e., 6328 Å Helium-Neon line) has limitations especially when the wavelength of use is considerably shorter, such as is the case of the water-window microscope systems. The use of higher sensitivity interferometric testing techniques, or the construction of an x-ray interferometer utilizing multilayer optics is becoming more essential due to the direct effect that system wavefront errors have on imaging quality. Existing techniques, such as multi-pass interferometry and holographic interferometry, have been used to increase the sensitivity of wavefront error detection.

These techniques must be improved as the wavelength shortens, especially in the area of aspheric system testing. The current designs that have been used involve spheres, but we are being considering systems employing aspheric mirrors with both moderate and severe aspheric departures, with unusual contours that are not easily tested. Multi-pass interferometry was used with great success on the MSSTA Ritchey- Chrétien telescope systems in assuring the accuracy of the systems prior to and supportive of other performance tests. It is also of extreme importance to employ these tests during the manufacturing of both the individual components as well as the final system. The use of multi-pass interferometry was made easier due to the highly reflective surface of the multi-layers even at the



longer test wavelength. This has made analysis more exact and the assessment of the performance at the shorter wavelengths has become more reliable.

## APPLICATIONS TO CANCER RESEARCH

Our current knowledge of tumor cell biology, detection and diagnosis has been made possible by steady improvements in microscopic methods for examining cells and tissues. Knowledge of cell ultrastructure made possible by high resolution transmission electron microscopy has revolutionized our concept of the organization of eukaryotic cells and the identification of organelles in the nucleus and cytoplasm. Recently, improvements in visible light instrumentation has led to the development of enhanced methods for the study of tumor cell growth and malignancy, and pre-malignant changes. For example, fluorescence microscopy, phase microscopy, differential interference contrast and polarizing microscopy have provided new approaches for research into pre-malignant changes and improvements in diagnosis. Moreover, the availability of low light level video cameras and computer enhancements of digitized images has led to improved resolution of the structures in motile cells. All current optical techniques are limited due to relatively low resolution and contrast of biological materials and the need, in most cases, for harsh fixatives, dyes and chemical additives.

The Water Window Imaging X-Ray Microscope has several potential features which could revolutionize tumor cell biology and cancer diagnosis. Its unique potential for detecting structures with spatial resolution in the object plane of 100 Å or better, along with its capacity to image living cells in aqueous, physiological environments is an advantage which is not available in any conventional microscopes. Although initial trials would be limited to cell monolayers in tissue culture, imaging could be extended to living tumor cells as well as analysis of frozen sections. In conjunction with tumor and cellular biology specialists, cell samples will be mounted and images obtained at x-ray wavelengths in the water window portion of the spectrum using high intensity synchrotron radiation sources. Computer analysis of the images will be carried out. These detailed laboratory/clinical tests and evaluation studies will provide the necessary data to establish the applicability of the Water Window Imaging X-Ray Microscope to cancer research.

## POTENTIAL FOR COMMERCIALIZATION

The successful completion of this project will result in the production of an entirely new imaging x-ray instrument which should permit the study of living tumor cells with unprecedented spatial resolution. It will allow the structures within the cells to be studied without the introduction of fluorescent dyes or chemical additives, which may alter the cellular processes under study. If the full optical potential of the microscope is realized, the *Water Window Imaging X-Ray Microscope* could become a standard diagnostic instrument in hospitals and medical centers throughout the world. It is envisioned that commercial instruments could be integrated with compact, internally contained, high intensity laser plasma x-ray sources. Its potential as an essential microscope for fundamental cell research would insure its commercial application in research centers and universities. Additionally the successful completion of this project will provide the impetus for the development of other complex advanced multilayer x-ray optical systems, of great importance to a broad range of other areas of science and technology.

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